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Diagnostic Uses of Amniocentesis in Late Pregnancy

Examination of amniotic fluid obtained by amniocentesis has become an established diagnostic procedure in the prenatal assessment of rhesus hæmolytic disease of the newborn. The biochemical composition of amniotic fluid and the appearances of the contained fœtal cells have been used to assess the age of the fœtus *in utero*. Further studies of its composition throughout pregnancy may reveal other aspects of fœtal functional development.

Rhesus Hæmolytic Disease of the Newborn (HDN)
Many methods have been described for determining the amount of bilirubin in amniotic fluid and assessing the degree to which the fœtus *in utero* is affected by HDN (Liley 1961, 1963, Freda 1965, Walker 1970). All such assessments must take into account that the bilirubin content of normal amniotic fluid decreases as pregnancy progresses; the highest levels of bilirubin encountered occur in normal pregnancies between 20 and 24 weeks' gestation, after which the concentration decreases until by term there is little or no detectable bile pigment. Thus assessment of severity of HDN is not based on the presence or absence of bilirubin but on whether the concentration is high for the stage of pregnancy, and whether the normal fall in content is occurring. Amniocenteses on two occasions 2–3 weeks apart are of more value than a single estimate. Values which are falling steadily usually mean a Coombs-negative infant or one only mildly affected *irrespective of the absolute levels*. A concentration which is maintained or, more gravely, increases means a correspondingly poor prognosis.

Assessment of Gestational Age
The biochemical composition of amniotic fluid changes as pregnancy progresses. The osmolality falls, due largely to the progressive decrease in sodium concentration, while the concentrations of urea and creatinine rise (Lind *et al.* 1969). The fœtal cells are scanty before 32 weeks and made up equally of basal and precornified types. Between 33 and 36 weeks the basal cells are replaced by precornified and later by cornified cells. The presence of anucleated squamous cells is characteristic of the term infant (Lind 1970).

Data have been presented showing a correlation between these biochemical and cytological changes and the length of gestation (Lind *et al.* 1971). A simple point scoring system for estimating gestational age has been devised based upon a knowledge of amniotic fluid true creatinine, the difference in urea concentration between amniotic fluid and maternal serum and finally the cytological features (Lind & Billewicz 1971). This scoring method allocates over 70% of cases to the correct gestational category when compared to the length of gestation calculated from menstrual dates. Of more interest is that the 'score age' correlates with the age determined by pædiatric assessment within 24 hours of delivery, in over 90% of cases. The scoring system thus offers the obstetrician the chance to assess fœtal age *in utero* in a manner which is highly relevant from the point of view of management after delivery.

Diagnostic Possibilities of Amniocentesis
Substances ranging from amino acids to enzymes and prostaglandins have been measured in amniotic fluid but few have been estimated serially throughout pregnancy. Before any diagnostic claims can be made the changing concentration and the range of normal values throughout pregnancy will have to be determined for each substance. The examples to be given have no diagnostic significance at the present time but show that various circumstances affecting the fœtus are reflected in the composition of amniotic fluid and reveal the possibility that fœtal functional development may be monitored *in utero*.

Hormones: Aleem *et al.* (1969) have described the changing amniotic fluid œstriol concentrations during normal pregnancy and how they are depressed with the increasing severity of rhesus hæmolytic disease.

Lipids: Total lipids are slightly increased during normal pregnancy due largely to an increase in the phospholipid fraction (Biezenski *et al.* 1968). This is of interest because the main phospholipid is lecithin which in turn is said to be responsible for 'surfactant'. Thus it may be possible to tell when a fœtus has sufficient lecithin present to reduce the chances of the respiratory distress syndrome developing.

5-HIAA: It has been reported that the concentration of 5-hydroxyindole acetic acid is increased in pregnancies complicated by pre-eclamptic toxæmia (Loose & Paterson 1966).

Amino acids: The amino-acid patterns of amniotic fluid samples in early pregnancy (Cockburn *et al.* 1970) and in late pregnancy (Levy & Montag 1969) are similar. The maternal urinary

amino-acid excretion changes markedly through pregnancy (F E Hytten, personal communication). On the assumption that the major source of amniotic fluid amino acids is fœtal urine it reveals a further aspect of fœtal autonomy.

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Dr Herbert E Reiss (*London*) said that in collaboration with Mr L J Butler, cytogeneticist at the Queen Elizabeth Hospital for Children, he had gained experience at Hackney Hospital in chromosome studies of fœtal tissues first from a study of spontaneous abortion cases and later from tissue culture of fœtal cells harvested at amniocentesis prior to termination of pregnancy by intra-amniotic hypertonic saline instillation (Butler & Reiss 1970).

Having elaborated a satisfactory technique they had embarked on a study of fœtal sex and chromosome abnormalities. This diagnostic service should be available to women with previous affected babies, to known carriers of chromosome translocation and to women over 40 years of age.

Maternal risk was negligible, but there were distinct fœtal risks, mainly isoimmunization and abortion. The main issues to be settled were:

(1) Route, vaginal or abdominal. Here he disagreed strongly with Dr Scrimgeour: the vaginal route carried a much greater risk (Riis & Fuchs 1966). Following the vaginal approach abortion had been described in other series and had occurred in one of his cases.
(2) Timing. He considered 14–15 weeks the best time: before this the transabdominal approach was difficult, the number of viable fœtal cells harvested was small, and the risk of spontaneous abortion (wrongly attributed by the patient to the amniocentesis) was greater.
(3) Liquor volume to be removed. The only constant factor about liquor volume in early pregnancy was its enormous variability (Reiss 1969). Valenti and Nadler (personal communication) removed about 45 ml amniotic liquor. He preferred to estimate uterine size bimanually and remove a volume dependent on the findings. Generally this was about 20 ml which proved safe and provided an adequate viable fœtal cell count.

The benefits of the procedure consisted not only in the elimination of the abnormal but also in the reassurance of the mother carrying a fœtus with a normal karyotype. However, the emotional stress on all involved in this type of work was great, particularly when the question of termination arose.

Finally Mr Reiss said the cytogeneticists had to answer the following questions before what was a pilot study could become a general screening programme: (1) What is the success rate in establishing fœtal cell cultures? (2) How reliable are the results? (3) How can the time gap between amniocentesis and result be reduced? (4) What is involved in terms of cost and personnel?

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Mr L J Butler (*Queen Elizabeth Hospital for Children, London E2*), in reply, said that despite their own figure of 95% and especially the average for all units of about 80%, in his opinion the success rate was not sufficiently high for large-scale random surveys on women over 35 because women in the 35–40 year age group had a less than 2% chance of carrying a fœtus with a chromosome abnormality and the risk of inducing abortion by amniocentesis could be as high as 0·5–0·7%. However, these figures were acceptable for women of exceptional reproductive age and for those of high genetic risk. In his experience failure was not due to poor or no growth of the primary cell cultures, infections or blood contamination, but almost entirely to a lack of good mitotic activity after a medium change or subculture. In this connexion, he hoped that a technique would become available to improve cell synchrony in the exceptional cultures. One hazard of the heavily blood-contaminated specimen was for partial clotting to occur in the container before it could be centrifuged, thus leading to substantial cell loss.

The different sera used were often implicated as a cause of culture failure. He had found that ordinary membrane-filtered calf serum from a reliable source was perfectly adequate; he had